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PROGRESS REPORT

Human Alveolar Macrophages and Emphysema

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PART I: Enzymes in man inhibited by alpha-1-antitrypsin

The goal of Part I is to locate enzymes in man which are inhibited by alpha-1-antitrypsin (AAT) and which may therefore produce emphysema in patients deficient in the inhibitor. The first part of the project was designed to investigate the human alveolar macrophage (AM) as a possible source of these enzymes. AM were examined first as a possible source of the causative enzyme(s) because they reside at the site of damage in emphysema.

AM were lavaged from lungs of subjects with normal lung function or from surgical specimens. Proteolytic activity was measured in cytosol by the method of Anson (1). Fluorescent antibody studies were performed by the indirect method of Weller and Coon (2) to determine whether AAT was present in AM.

AM extracts contain maximal protease activity at pH 3.0 (range 2.0-6.0) (fig. 1). The protease(s) is inhibited by purified AAT when measured at pH 4.0.

Fluorescent antibody studies showed that there is AAT in AM. The fluorescence was blocked with unlabeled anti-rabbit gamma globulin and did not occur when cells were treated with anit-human albumin or with normal rabbit gamma globulin instead of anti-AAT. Fluorescence of AM from one patient with normal lung function and a deficiency of AAT (Pi Type ZZ) was minimal, but when the cells were incubated with normal serum before staining, the fluorescence was enhanced.

These studies demonstrate the presence of AAT-inhibitable proteases in AM, and show that AM can concentrate AAT from fluid-phase. Although the relationships between AM protease(s) and AAT require further exploration, AM could provide a source of the protease activity that engenders the emphysema of AAT-deficient subjects.

The studies on AM were facilitated by the development of improved methods for purifying AM from surgical specimens. Two methods of purification were tried. Sedimentation at 1 x g of fluid recovered from lung lavages was followed by erythrocyte lysis. The populations of AM which resulted were more than 96% pure by morphologic and phagocytic criteria. This new method permitted me to employ large numbers of replicates in studies of macrophage

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cathepsins. In addition, sedimentation through a dense aqueous solution of sodium diatrizoate and ficoll (density 1.07 gm/ml) resulted in a band of cells containing 66% (about a 10 fold purification) of foamy macrophages similar to those I have previously found in lungs distal to obstructed bronchi (3). Large amounts of surface active material were documented in this fraction.

PART II: Biochemistry of alpha-1-antitrypsin

Earlier progress reports provided evidence that trypsin and chymotrypsin compete for active sites on alpha-1-antitrypsin (AAT). A recent experiment has allowed me to split the trypsin and chymotrypsin inhibiting functions of AAT thereby proving that the inhibiting sites are overlapping but not identical.

Phenylglyoxal hydrate has been shown to block arginyl residues as long as the protein is maintained in an environment which contains an excess of the blocking reagent (4). Therefore, the following experiment was performed: Phenylglyoxal hydrate was added to AAT. After a suitable incubation period the treated and control samples of AAT were passed through identical sephadex G25 columns to remove the unbound reagent, and the inhibitor was immediately tested for anti-tryptic and anti-chymotryptic activity. The samples were then incubated at 37° C for 48 hours while being dialyzed against buffer. These conditions are sufficient to remove the phenylglyoxal hydrate from the arginyl residues. After dialysis the samples were again tested for anti-tryptic and anti-chymotryptic activity.

The results showed that blocking arginyl residues eliminated anti-tryptic but not anti-chymotryptic activity and the anti-tryptic activity was restored when arginyl residues were unblocked (Table 1). These results improve on my earlier arginine blocking experiments in two ways:

1. The mild unblocking conditions allow regeneration of activity. These results prove that the blocking reagent did not denature the AAT.

2. Splitting of anti-tryptic and anti-chymotryptic functions by blocking arginyl residues confirms that there are two overlapping inhibitory sites on AAT and suggests that the mechanism of inhibition is strongly related to the amino acid specificity of the inhibited enzyme.

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3. Cohen, A.B. and M.J. Cline: Am. Rev. Resp. Dis. 106:69, 1972.

4. Takahashi, K.: J. Biol. Chem. 243:6171, 1968.

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Figure

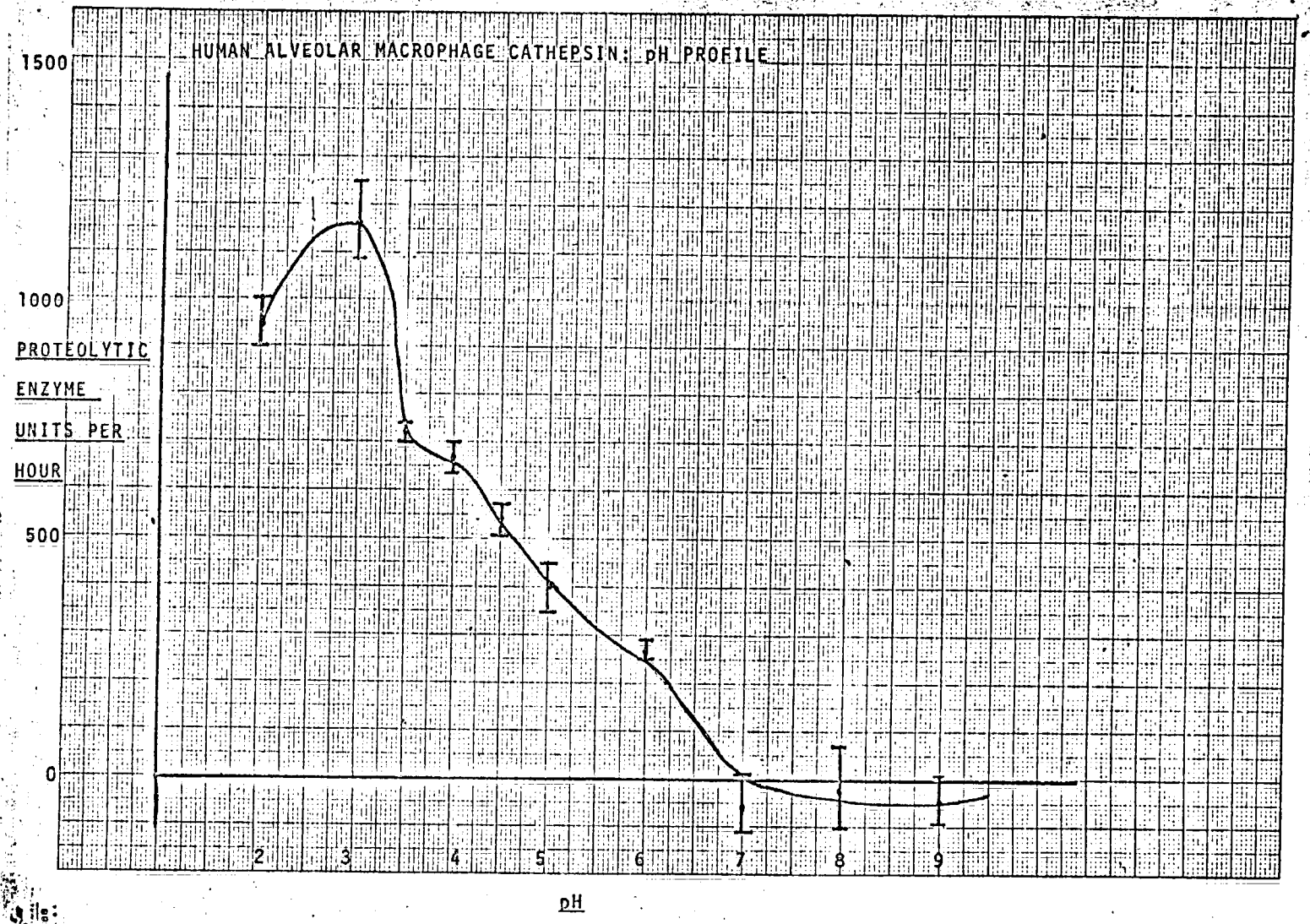

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TABLE I

Effect of Modification of Arginyl Residues on α-1-Antitrypsin Activity

	Enzyme	α-1-Antitrypsin + phenylglyoxal hydrate + enzyme	% Inhibition of enzyme by modified α-1-antitrypsin	α-1-Antitrypsin + enzyme	% Inhibition of enzyme by unmodified α-1-antitrypsin
BAPNA (trypsin substrate)					
1. Mean ΔOD/30 min	.368	.433	0*	.278	24.3†
S.E.	.008	.004		.013	
2. Mean ΔOD/30 min	.337	.347	0*	.236	29.9†
S.E.	.003	.008		.009	
BTEE (chymotrypsin substrate)					
1. Mean ΔOD/min	.069	.043	37.7‡	.051	26.1†
S.E.	.008	.002		.002	
2. Mean ΔOD/min	.064	.040	36.7†	.049	23.4†
S.E.	.001	.000		.000	
BAPNA-After PGH Removal					
1. Mean ΔOD/30 min	.447	.324	27.5†	.309	30.9†
S.E.	.010	.004		.002	
2. Mean ΔOD/30 min	.437	.238	45.5†	.324	27.5†
S.E.	.019	.006		.013	

Levels of statistical significance using Student's t-test:

*>0.2, †<0.001, ‡<0.025

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